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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/18</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/14081</b> <b>(43) International Publication Date:</b> 17 May 1996 (17.05.96)
<b>(21) International Application Number:</b> PCT/NL95/00370 <b>(22) International Filing Date:</b> 26 October 1995 (26.10.95) <b>(30) Priority Data:</b> 94203205.3 3 November 1994 (03.11.94) EP <b>(34) Countries for which the regional or international application was filed:</b> AT et al. <b>(71) Applicant (for all designated States except US):</b> BOEHRINGER MANNHEIM GMBH (DE/DE); Sandhofer Strasse 116, D-68298 Mannheim (DE). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> SWAAK, Anthonius, Josef, Gerardus [NL/NL]; Kralingseweg 322, NL-3066 RB Rotterdam (NL). <b>(74) Agent:</b> SMULDERS, Th., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).		<b>(81) Designated States:</b> AU, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KR, LT, LV, MD, MX, NO, NZ, PL, RU, SI, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> USE OF ERYTHROPOIETIN IN THE TREATMENT OF RHEUMATOID ARTHRITIS  <b>(57) Abstract</b>  A novel use of the known protein Erythropoietin (EPO) and/or a derivative and/or a fragment thereof is disclosed. EPO is used as a pharmaceutical for the treatment of chronic inflammations. A particularly beneficial result is seen in patients suffering from rheumatoid arthritis (RA). Significant effects are seen in clinical variables such as morning stiffness, swollen joints, and the like.		

ATTORNEY DOCKET NUMBER: 10165-022-999  
SERIAL NUMBER: 10/612,665  
REFERENCE: B18

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Title: Use of erythropoietin in the treatment of rheumatoid arthritis.

The invention relates to certain novel uses of the known protein erythropoietin (EPO), or substances having such activity as disclosed herein.

Erythropoietin is a humoral regulator of erythropoiesis, which stimulates the production of erythrocytes. In normal conditions it is produced in sufficient quantities in the kidneys and the liver.

In case of hypoxic shocks (such as massive blood loss) erythropoietin production needs to be increased, which means that it has to be synthesised de novo. In disease-free conditions, erythropoietin levels in circulation are extremely low.

Certain diseases or side-effects of treatments of certain diseases lead to a chronic anaemia which overcharges the capacity of erythropoietin production, or otherwise cannot be met by the body's own erythropoietin resources. These diseases include chronic insufficiency of the kidneys, anaemias associated with malignancies, neonate anaemia, chronic anaemia associated with rheumatoid arthritis (ACD), anaemia after bone marrow transplantation, aplastic anaemia, myeloplastic syndrome and various haemoglobin related diseases. Also anaemic side effects have been shown to occur in various chemotherapies and AZT-therapy.

In these cases it may be helpful to administer EPO to increase erythrocyte production.

Human EPO is available as a recombinant protein, which ensures that sufficient quantities can be produced in a very pure form.

Several studies with recombinant human erythropoietin (r-hu-Epo) have been carried out, mainly in patients who underwent renal dialysis for chronic renal failure, in which diminished production of Epo and severe anaemia requiring regular bloodtransfusions occurs. A correction of anaemia by

r-hu-Epo was shown in these cases with minimal side-effects (16,17,18). In AIDS-patients treated with Zidovudine, causing bone marrow suppression, administration of 100 U r-hu-Epo/kg thrice weekly intravenously, significantly decreased  
5 transfusion requirements (19).

The invention provides a novel use of erythropoietin which is not directly related to its erythrocyte stimulating properties.

This use is specifically clear in rheumatoid arthritis, which  
10 therefore is more specifically described as explanatory example for the invention.

Rheumatoid arthritis is an inflammatory disease of synovial membranes, usually expressing itself in a symmetrical polyarthrititis. During the course of their disease 70% of  
15 rheumatoid arthritis (RA) patients develop some kind of anaemia (1), which may be due to iron deficiency (2,3), vitamin B12 deficiency or folic acid deficiency (4,5), haemolysis or adverse reactions to anti-rheumatic drugs (6,7). In addition active RA is frequently (in nearly 50%)  
20 accompanied by anaemia of chronic disease (ACD) (8).

Factors involved in the pathogenesis of ACD are ineffective erythropoiesis (9), interleukin-1 (10), tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) (11), decreased erythropoietin synthesis (5,12,13) and/or a decreased response to  
25 erythropoietin by the bone marrow (14,15).

So far only a few studies with r-hu-Epo have been carried out in RA patients. A haemoglobin (Hb) rise was shown in two anaemic RA patients treated with r-hu-Epo, 125-250 IU/kg thrice weekly, a significant haematocrit rise was recorded  
30 (20).

We have treated ten RA patients who suffered from ACD with recombinant human EPO.

In all RA patients a rise in haemoglobin was observed. Despite a wide range of values, the increase in haemoglobin  
35 became significant after the second week of treatment with recombinant human EPO.

Besides this expected result of EPO treatment a different unexpected benefit was obtained by the treatment.

The invention thus provides the use of erythropoietin or a substance having erythropoietin-like activity in the  
5 preparation of a pharmaceutical for the treatment of chronic inflammations, especially those related to (auto-)immune diseases, in particular RA. In RA we found an overall improvement in the clinical parameters for scoring disease activity. Most impressive are the results on clinical  
10 variables such as painscore and morning stiffness as disclosed below. A significant decrease in the number of tender joints was already observed after two weeks of treatment. The changes in other clinical parameters did not reach statistical significance due to the wide range of values and the small  
15 number of patients in the study. However, when the parameters were expressed as percentages of their baseline value, significant improvements were observed.

In addition to this effect on clinical variables a further positive effect was seen in the area of an overall  
20 sense of well-being of the treated patients.

According to the invention any erythropoietin which has the ameliorating effect on chronic inflammations can be used. Preferably this erythropoietin is not immunogenic so that it can be administered repeatedly. This will usually lead to the  
25 use of human erythropoietin of any origin, although recombinant erythropoietin seems the product of choice because of its purity and constant quality. On the other hand it may very well be possible to use non-human truncated forms of mammalian erythropoietin as long as they have the activity and  
30 are not immunogenic upon normal administration to patients. Selected mutants (longer acting, more stable), fragments or derivatives of erythropoietin may also be used as long as they fulfil both criteria.

It is worthwhile to note that patients not having a kind  
35 of anaemia can thus be treated with EPO. However, caution has to be taken that Hb-levels do not rise to detrimental levels. Ways of lowering the Hb-levels are well-known in the art.

Also, it will be necessary to ensure that no hypertension occurs at a detrimental level. Ways to avoid such a reaction are also well known in the art.

One of the mechanisms through which EPO may ameliorate the disease symptoms in RA (or other chronic inflammations) is that it mobilises iron towards haemoglobin production. Iron (free and/or bound in ferritin) deposits are known to occur in the synovia of RA-affected patients. Synovial fluid iron levels correlate with RA activity and therefore it is thought that iron is involved in the initiation or maintenance of RA synovitis through mediating tissue damage. The role of iron in the pathogenesis of RA may be related to the fact that iron stimulates the production of hydroxyl radicals, which are very potent agents in the destruction of cartilage, membranes and proteins. A thorough discussion of the role and the mechanisms of iron in the inflamed joint can be found in Vreugdenhil et al. (23). In said study it is suggested to administer iron chelators to RA patients. EPO does not chelate iron. However, EPO does mobilise iron to be incorporated into haemoglobin through serum transferrin. Thus EPO may reduce the levels of iron in the synovial fluids.

Another possible mechanism which may be responsible for the unexpected beneficial effect of EPO in (especially) RA, may be found in its influence on the  $T_H1/T_H2$  balance.

One of the key functional parameters determining the outcome of immune responses, for example infectious agents, is the nature of the cytokines produced locally by immune cells. At this moment evidence is obtained that T-cells can be classified into  $T_H1$  and  $T_H2$  cells; both characterized by a different cytokine secretion profile.  $T_H1$  cells secrete IL-2 and TNF- $\gamma$  upon activation but not IL-4 or IL-5, and  $T_H2$  cells produce IL-4 and IL-5 but not IL-2 or TNF- $\gamma$ . The differential cytokine profile of these CD4+T cells correlates with different effector functions exerted by these cells:  $T_H1$  cells mediate delayed type hypersensitivity (DTH) responses and  $T_H2$  provide superior help for antibody productions by B cells. There is also some support for the notion that  $T_H1$  and  $T_H2$

cells are progeny of Th<sub>0</sub> cells which can produce IL-2, TNF- $\gamma$ , IL-4 and IL-5 simultaneously. Th<sub>1</sub> like cytokine secretion profile. In different animal studies and observations in human diseases, like leprosy, evidence is obtained that the balance  
5 between Th<sub>1</sub> and Th<sub>2</sub> response determined the outcome of for example an infectious disease and disease manifestations. At this moment a selective activation of Th<sub>1</sub>-like T cells is proposed as a hallmark of the aetiopathogenesis of rheumatoid arthritis. Evidence for this hypothesis is formed by the fact  
10 that on histopathological examination of the synovial tissue, a DTH like of inflammatory reaction is observed which is characteristic for a Th<sub>1</sub> response.

Some observations in our RA patients treated with r-hu-EPO showed a rise in serum IgE levels; which is consistent  
15 with the concept that EPO can give support for a Th<sub>2</sub>-like response. In other ways influencing the Th<sub>1</sub>-Th<sub>2</sub> balance in a more Th<sub>2</sub> cytokine secretion profile. Indirect evidence for this hypothesis is formed by the fact that 2 out of 3 monoclonals raised against EPO are of the IgE class (IgE synthesis is  
20 regulated by IL-4).

When EPO is administered to new-born rats a reduced neutrophil production is observed. This reduced neutrophil production may be partly responsible for the ameliorating effect observed in our patients in that neutrophils play a key  
25 role in inflammatory reactions.

It has also been observed that EPO can in some ways counteract the activity of TNF- $\alpha$ . TNF- $\alpha$  is an important pro-inflammatory cytokine.

It may also be the case that EPO diverts the multipotent  
30 progenitor blood cells to the production of erythrocytes instead of granulocytes.

#### EXPERIMENTAL

##### Patients:

This study focused on the effects of r-hu-Epo on RA  
35 disease activity parameters. It is a part of a project studying the pathogenesis of ACD and possible therapeutic

strategies. The effect of r-hu-Epo on the anaemia and iron metabolism is reported in more detail (21).

Ten patients with RA (22) were studied, fulfilling the criteria for ACD as proposed by Carwright (8). ACD was confirmed by measuring stainable iron in a bone marrow preparation. Patients treated previously with iron, vitamin B12, folic acid and cytotoxic drugs were excluded. Other causes of anaemia were also excluded such as the presence of haematuria, positive occult bloodtest in stool, decreased creatinine clearance, haemolysis and low vitamin B12 or folic acid.

The demographic features of the studied patients are summarized in table I. All patients used a variety of non steroidal anti-inflammatory drugs.

Treatment:

Recombinant human Erythropoietin (r-hu-Epo, Boehringer, Mannheim, Germany), was administered three times a week at a dose of 240 units/kg subcutaneously at the right upper leg for 6 weeks.

Clinical and laboratory monitoring:

Detailed clinical and laboratory evaluation was performed at entry and weekly by the same physician, till the end of the study (6 weeks), then at 9 and 12 weeks after onset of the study. Routine laboratory procedures were used for assessment of haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpus haemoglobin (MCH) and reticulocytes count. Serum iron was measured spectrophotometrically (Instruchemie, Hilversum, the Netherlands). Transferrin and CRP was assessed with a nephelometer (Ablon Medical Systems, Leusden, the Netherlands) and serum ferritin by solid phase enzyme immune assay (Ferrizyme, Abbott Labs, Chigaco, USA). The erythrocyte sedimentation rate (ESR) was measured by the Westergren method. The Ritchie index, grip strength, number of swollen joints, morning stiffness and a subjective pain score (visual analogue scale, 0-10 points) were assessed as well. Liver and kidney function tests were performed to monitor possible side effects.



Data evaluation:

- For evaluation all clinical data were stored and analyzed on a Wang personal computer using the Lotus-1-2-3 program.
- 5 Statistical evaluation of the results was by Fishers' exact test for group differences. P values of 0.05 or less were considered significant.

**RESULTS**Effect of r-hu-Epo on the anemia of chronic disease (ACD).

- 10 In all RA patents a rise in haemoglobin was observed (table II). Despite of the wide range of values, the increase in haemoglobin became significant after the second week of treatment compared to baseline values. When treatment was stopped haemoglobin stayed significant higher compared to the
- 15 baseline value, but dropped in the 12th week.

Iron deficiency developed as shown by the fact that five patients were characterized by ferritin levels lower than 40 µg/ml.

Effect of r-hu-Epo on disease activity parameters.

- 20 Laboratory parameters: ESR and CRP.

- A decrease in ESR was found in all patients (table III), which started at the third week of treatment and remained so until the end of the study. As illustrated the decrease in eight patients was more than 20% of their baseline value;
- 25 which was highly significant. The same holds true for the CRP values, but due to the wide range in the absolute values and small number of investigated patients, no significance could be calculated. However, expressing the values as a percentage of the baseline value, also in this way after the third week
- 30 of treatment, a significant decrease in the CRP levels was observed.

Subjective clinical scores: painscore (PS) and morningstiffness (MS).

- Both parameters (PS and MS) showed during the follow-up a
- 35 tendency to decrease (table IV). Caused by the variability in absolute values and small number of patients a significance could not be calculated. When the values were expressed in a

percentage of the baseline value, the PS decreased significantly after the third week of treatment and the MS after the sixth week.

Objective disease activity scores: gripstrength (GS).

5 Ritchie Index (RI) and number of swollen joints (SJ).

10 All parameters as shown in table V showed a continuous tendency towards improvement which lasted during, and also after, the treatment period. In the absolute changes in number of tender joints a significant decrease could be calculated from the third week of treatment. Also a continuous decrease in the number of swollen joints was observed from T3 on and at T9 nine out of ten patients had less swollen joints, which was highly significant.

15 Caused by the variation of the individual values of the GS, it was impossible to calculate a significance. However, when the values were expressed as a percentage of their baseline values after three weeks of treatment, a significant increase in GS was noted. It should be mentioned that the GS remained stable in three patients during our investigation.

TABLE I

Demographic features of ten patients characterized on having anaemia of chronic disease (ACD) and rheumatoid arthritis (RA)

Female/Male	9/1
Mean age (years)	68 ± 6,5
Treatment:	
Prednisolone	(2 patients) 5 mg
Sulphasalazine	(3 patients) (range) 1.5-2.5 g/day
Plaquenil	(1 patient) 200 mg/day
Auromyose	(1 patient) 50 mg/in 2 weeks
D-Penicillamine	(2 patients) (range) 500-750 mg/day

- 5 All patients were treated for more than 2 months with the mentioned disease modifying anti-rheumatic drugs.

TABLE II

Effect of recombinant human erythropoietin (r-hu-Epo) therapy on haemoglobin and ferritin levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

5

Variable	Base-line	Values during the 6 weeks therapy and after 3 and 6 weeks of treatment.							
	TO*	T1	T2	T3	T4	T5	T6	T9	T12
Hemo-globin	5.9	6.1	6.5**	6.8	7.0	7.2	7.2	7.2	6.6
mmol/l	0.4	0.5	0.6	0.7	0.9	1.0	1.0	1.1	0.9
± sd									
Ferritin material	216		143**				80	49	61
µg/ml	140-318		44-301				14-157	19-82	52-84
Range									

\* Refers to treatment weeknumber.

\*\* Marks the treatment period when the differences between baseline became significant.

10

TABLE III

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
ESR (mmH) mean ranges	82 42-137	61** 18-112	53** 7-98	56** 7-111
ESR (%) mean ranges	100 -	63 32-107	59 16-108	64 16-144
Number of patients with a change > 20% baseline value	-	8**	7**	8**
CRP (mg/l) mean ranges	51 10-105	45 4-113	43 3-122	44 1-144
CRP (%) mean ranges	100 -	85 17-155	85 8-204	81 5-181
Number of patients with a change > 20% baseline value	-	5**	6**	6**

\* Refers to treatment weeknumber.

10 \*\* Marks the treatment period when the differences compared to baseline values became significant.  
P > 0.05, Fishers's exact test.

TABLE IV

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the overall pain score (PS) and morning stiffness duration (MS) at the defined time periods after onset treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
PS				
mean	3.9	3.0	2.7	2.8
ranges	2.7	1-5	1-5	1-5
PS (%)				
mean	100	82	70	73
ranges	-	50-150	33-150	33-100
Number of patients with a change > 20% baseline value	-	7**	8**	6**
MS (min)				
mean	45	37	35	36
ranges	10-120	10-120	10-120	10-120
MS (%)				
mean	100	88	78	85
ranges	-	50-150	50-150	50-150
Number of patients with a change > 20% baseline value	-	3	5**	5**

\* Refers to treatment weeknumber.

\*\* Marks the treatment period when the differences compared to baseline values became significant.  
P > 0.05, Fishers's exact test.

TABLE V

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the Ritchie index (RI), number of swollen joints (SJ) and grip strenght (GS) at the defined time periods after onset treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
RI mean	13	10.2	7.7**	6**
RI ranges	3-38	1-22	1-14	2-13
RI (%) mean	100	66	62	56
RI ranges	-	25-100	33-111	22-95
Number of patients with a change > 20% baseline value	-	8**	7**	9**
SJ mean	8	6	4.5	4.5
SJ ranges	6-5	3-11	2-8	1-9
SJ (%) mean	100	72	61	51
SJ ranges	-	42-100	37-100	20-100
Number of patients with a change > 20% baseline value	-	8*	7*	9*
ESR (mmH) mean	72	87	91	90
ESR ranges	15-190	20-220	20-220	15-220
ESR (%) mean	100	112	118	118
ESR ranges	-	90-133	90-166	90-166
Number of patients with a change > 20% baseline value	-	4**	4**	5**

\* Refers to treatment weeknumber.

10 \*\* Marks the treatment period when the differences compared to baseline values became significant.

P > 0.05, Fishers's exact test.

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CLAIMS

1. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of chronic inflammations.
2. Use according to claim 1, wherein the inflammation is  
5 associated with an immune disease.
3. Use according to claim 2 wherein the immune disease is an auto-immune disease.
4. Use according to claim 3, wherein the auto-immune disease is rheumatoid arthritis.
- 10 5. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of symptoms associated with rheumatoid arthritis.
6. Use according to claim 5, wherein the symptoms treated  
15 comprise at least one of the group of morning stiffness, painful and swollen joints, loss of grip strength and pain.
7. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the amelioration of disease activity of  
20 rheumatoid arthritis.
8. Use according to anyone of the afore going claims, wherein the erythropoietin is human erythropoietin.
9. Use according to anyone of the foregoing claims wherein the erythropoietin or the substance having such activity is of  
25 recombinant origin.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/NL 95/00370

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 171 304 (CHUGAI SEIYAKU K.K.) 28 August 1986 see the whole document ---	7-9
Y	EP,A,0 269 394 (KIRIN-AMGEN, INC.) 1 June 1988 see page 2, line 5 - line 23; claims 1-4 see page 2, line 33 - line 39 see page 2, line 45 - line 47 --- -/--	1-9

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search

30 January 1996

Date of mailing of the international search report

15. 03. 96

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# INTERNATIONAL SEARCH REPORT

Intern    nal Application No  
PC1/NL 95/00370

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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**INTERNATIONAL SEARCH REPORT**

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